Altered chicken thyroid hormone metabolism with chronic GH enhancement *in vivo*: consequences for skeletal muscle growth

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Abstract

In contrast to most vertebrates, GH reportedly has no effect upon somatic growth of the chicken. However, previous studies employed only one to two dosages of the hormone, and limited evidence exists of a hyperthyroid response that may confound its anabolic potential. This study evaluated the effects of 0, 10, 50, 100 and 200 µg/kg body weight per day chicken GH (cGH) (0–200 GH) infused i.v. for 7 days in a pulsatile pattern to immature, growing broiler chickens (9–10 birds/dosage). Comprehensive profiles of thyroid hormone metabolism and measures of somatic growth were obtained.

Overall (average) body weight gain was reduced 25% by GH, with a curvilinear, dose-dependent decrease in skeletal (breast) muscle mass that was maximal (12%) at 100 GH. This profile mirrored GH dose-dependent decreases in hepatic type III deiodinase (DIII) activity and increases in plasma tri-iodothyronine (T_3), with both also maximal (74 and 108% respectively) at 100 GH. No effect on type I deiodinase was observed. At the maximally effective dosage, hepatic DIII gene expression was reduced 44% versus controls. Despite dose-dependent, fold-increases in hepatic IGF-I protein content, circulating IGF-I was not altered with GH infusion, suggesting impairment of hepatic IGF-I release. Significant, GH dose-dependent increases in plasma non-esterified fatty acid and glucose, and overall decreases in triacylglycerides were also observed. At 200 GH, feed intake was significantly reduced (19%; $P<0.05$) versus controls; however, additional control birds pair-fed to this level did not exhibit any responses observed for GH-treated birds.

The results of this study support a pathway by which GH impacts on thyroid hormone metabolism beginning at a pretranslational level, with reduced hepatic DIII gene expression, translating to reduced protein (enzyme) expression, and reflected in a reduced level of peripheral T_3-degrading activity. This contributes to decreased conversion of T_3 to its inactive form, thereby elevating circulating T_3 levels. The hyper-T_3 state leads to reduced net skeletal muscle deposition, and may impair release of GH-enhanced, hepatic IGF-I.

In conclusion, GH has significant biological effects in the chicken, but profound metabolic actions predominate that may confound positive, IGF-I-mediated skeletal muscle growth.

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Introduction

Growth hormone (GH) is considered essential for postnatal somatic growth in virtually all vertebrate species as diverse as fish (Sato *et al.* 1988, Rahman *et al.* 1998) and domestic mammals (for review see Etherton & Bauman 1998). The magnitude of this effect is greatest for teleosts, where transgenic models exhibit over 13-fold larger body size than non-transgenic controls (Du *et al.* 1992).

Among all species in which GH action has been studied, there are two apparent anomalies that have escaped conclusive explanation. The guinea pig has long been recognized as exhibiting GH-independent growth, in so far as hypophysectomy does not reduce growth rate, and pituitary extracts do not affect growth of either hypophysectomized or pituitary-intact animals (Mitchell *et al.* 1954, Clayton & Worden 1960, Baumann 1997).

A less-recognized aberrant GH model is the domestic chicken. A general conclusion frequently reported in the literature is that exogenous GH has no effect on the growth of modern, commercial strains of meat-type chickens (i.e. broiler), and this is frequently attributed to

the speculation that the modern broiler has reached its genetic potential for maximal growth. However, exogenous GH fails to improve growth even in slow-growing, random-bred lines that have not been subjected to intense genetic selection for growth (Peebles *et al.* 1988).

A hallmark of the anabolic effect of GH in all GH-responsive species is increased skeletal muscle growth, mediated via the action of insulin-like growth factor-I (IGF-I). Studies employing pituitary-derived, as well as recombinant chicken GH (cGH) administered to pituitary-intact birds, have failed to demonstrate a consistent, significant increase in circulating IGF-I, or significant positive muscle growth response in immature, growing broilers (for review see Vasilatos-Younken 1999). Limiting the scope of these studies have been practical constraints in experimental design, most importantly that, in all cases, only one or two dosages of the hormone have been employed. Previous studies from our laboratory have also demonstrated that, at GH dosages at which significant metabolic responses have been obtained, evidence of a hyperthyroid effect of GH has been observed, which may limit the anabolic potential of the hormone (for review see Vasilatos-Younken & Scanes 1991).

In the present study, a comprehensive GH dose–response study was conducted in which recombinant cGH was administered in a physiologically appropriate manner (pulsatile, intravenous infusion) to sexually immature, growing, broiler chickens during the period of late post-hatch development. In addition to measurement of somatic growth (body weight gain, longitudinal bone growth, skeletal muscle mass, etc.), comprehensive assessment of components of the thyroid hormone axis were evaluated to provide a more complete profile of potential changes in thyroid hormone metabolism attendant with GH enhancement. It was our hypothesis that GH would elicit a positive growth response, including a measurable increase in skeletal muscle deposition, at a lower dosage than previously utilized. At higher dosages, the metabolic effects of the hormone (as reflected by alterations in thyroid hormone metabolism) would predominate, which might limit further the anabolic potential of GH.

Materials and Methods

All animal procedures were approved by Pennsylvania State University Institutional Animal Care and Use Committee under protocol no. 89R1389G197.

Female broilers were surgically prepared with jugular vein catheters for chronic, intravenous delivery of cGH (Cravener & Vasilatos-Younken 1989). When birds were 8 weeks of age (late post-hatch, juvenile), cGH was infused in a pulsatile pattern for 7 days at one of five dosages (0, 10, 50, 100 or 200 g/kg body weight (BW) per day) (ten birds/dosage) and feed intake (FI) was monitored daily. The infusion protocol has been described previously (Vasilatos-Younken *et al.* 1988), and is based on a pulse interval (90 min) and duration (60 min) that replicate the endogenous pattern of GH secretion in sexually immature, growing broilers. To control for possible contributions of reduced FI on measured responses, pair-fed groups for GH treatments were established. Pair-feeding of vehicle-infused birds was accomplished by restricting their feed to the identical level of that consumed by the GH treatment groups; the latter group exhibited significantly reduced FI in comparison to controls.

Animal studies

Animals All birds used in these studies were immature, broiler-strain females reared at Pennsylvania State University, Poultry Education and Research Center, and maintained under a 16-h light:8-h darkness cycle throughout. Birds were fed a high-protein, micronutrient-fortified broiler feed (30% crude protein, 0·78% methionine, 1·57% lysine, 3200 kcal metabolizable energy/kg, calculated analysis), and water was provided *ad libitum* from hatching through termination of the studies, except prior to surgery and during pair-feeding as indicated.

Surgical procedures At 6 weeks of age, birds were acclimated to individual cages in a temperature- and light-controlled room for 6 days. Feed and water were then withheld for 12–15 h in preparation for surgical catheterization. Birds were anesthetized with sodium pentobarbitol (65 mg/ml; 0·56 ml/kg BW total dosage, i.v.). Catheters were inserted into the right jugular vein as described previously (Cravener & Vasilatos-Younken 1989). Each catheterized bird was maintained in a harness/spring tether/fluid swivel system to allow for unrestricted, 360° movement, and connected to a microprocessor controlled infusion pump (Model AS20A; Travenol Laboratories, Hookset, NH, USA) (Cravener & Vasilatos-Younken 1989). Birds were allowed 3–6 days of postoperative recovery, and normal, pre-surgical behavior, FI and BW gain were confirmed prior to initiation of infusions at 8 weeks of age. Infusions were conducted in two experimental replicates over time, approximately 3 weeks apart, with every treatment included in each experiment.

Dosage calculations and treatment administration Over the developmental period prior to sexual maturity, plasma GH concentrations in broilers similar to those used in the present study can range from <10·0 ng/ml (baseline troughs) to over 100 ng/ml (secretory peaks) (Vasilatos-Younken & Zarkower 1987, Johnson 1988, R Vasilatos-Younken, unpublished data). A series of dosages (0, 10, 50, 100 and 200 µg/kg BW per day) was chosen that was calculated to result in circulating concentrations over this range, and to include the approximate dosage of cGH that was estimated in a previous study to be

maximally effective in terms of metabolic responses (123 µg/kg BW per day; Vasilatos-Younken *et al.* 1988). Birds were selected to be 8 weeks of age at the initiation of infusions, because at this time endogenous GH secretion is low and circulating concentrations are barely detectable (<1·0 ng/ml). This was to avoid superimposing exogenous hormone pulses on pre-existing (endogenous), high-amplitude secretory peaks, as would occur at very young ages. It was felt that the latter scenario would result in a refractory state, characterized by responses suggestive of a lack of GH action, as reported previously (Moellers & Cogburn 1995).

The cGH preparation used was recombinantly derived (Lucky Biotech, Seoul, Republic of Korea) and contained <20 endotoxin units/mg bacterial endotoxin. It was identical in immunological potency to a pituitary preparation purified in our laboratory and previously described (lot no. RVY03; Cravener & Vasilatos-Younken 1989). The biological potency of the recombinant preparation in a hypophysectomized rat bioassay was 0·8 IU/mg as measured by weight gain, and 1·2 IU/mg as measured by widening of the epiphyseal growth plate, which are also similar to values for the pituitary preparation, as previously reported (Cravener & Vasilatos-Younken 1989). GH was dissolved in a bicarbonate-buffered saline solution containing 0·025 M $NaHCO_3$ and 0·025 M Na_2CO_3, pH 9·4, plus 0·1% chicken serum albumin. Infusion pumps delivered either the appropriate GH solution or vehicle (buffer as described above) over a 2-min period every 90 min, 24 h/day (0·8 ml total volume/bird per day) for 7 consecutive days. Thus, 16 pulses or peaks per 24-h period were produced. New, disposable sterile syringes containing fresh cGH and vehicle solutions were loaded into the pumps daily.

Data collection FI was measured daily throughout the study. Blood samples were collected by brachial venipuncture on the final day of infusion, immediately following a GH pulse, to determine GH peak amplitudes achieved by infusion. Heparinized blood samples were placed on ice and processed immediately. Plasma was obtained by centrifugation, aliquoted to multiple vials, and stored at −80 °C until assayed. The patency of each intravenous catheter was verified at the termination of infusion, and any bird for which the catheter was not intact and functioning was eliminated from the data set. Animals were killed by rapid decapitation and organs and tissues excised immediately (Nickel *et al.* 1977). Liver samples were rapidly frozen in liquid nitrogen and stored at −80 °C for later analysis.

Plasma hormones and metabolites

Plasma GH was determined by a homologous cGH radioimmunoassay (RIA) as previously described (Vasilatos-Younken 1986). Plasma thyroxine (T_4) and tri-iodothyronine (T_3) were measured as previously described (Vasilatos-Younken *et al.* 1997). Plasma insulin, IGF-I and IGF-II were measured as described by McMurtry *et al.* (1983, 1994, 1998), and tissue IGF-I was measured as described by Rosselot *et al.* (1995). McMurtry *et al.* (1994) demonstrated that chicken IGF-binding proteins (IGFBPs) are acid-labile, so that interference of IGF-I detection by IGFBPs is not a problem using the indicated method of acid/ethanol extraction. Plasma glucagon was determined as previously described (Allen & McMurtry 1984) using reagents purchased from Linco Research, Inc. (St Charles, MO, USA). Plasma glucose, triacylglycerides, uric acid (Bulletin nos 16UV, 335-B, and 685; Sigma Chemical Co., St Louis, MO, USA), and non-esterified fatty acids (NEFA) (NEFA-C; Wako Chemical Co., Dallas, TX, USA) were determined with commercially available kits.

Muscle free 3-methyl histidine content

A subsample (40–50 g) of breast muscle was homogenized to the consistency of fine talcum powder by grinding frozen samples together with dry ice in a stainless steel micro-mill. The dry ice was allowed to sublimate and 100–200 mg of the frozen powder was extracted with 2·5 ml 2% perchloric acid (PCA). Muscle free 3-methyl histidine was determined in PCA extracts by the method of Wassner *et al.* (1980), using 3-ethyl histidine as an internal standard.

Hepatic deiodinase activity

For determination of hepatic type I (DI) and III (DIII) iodothyronine deiodinase activities, liver microsomal fractions were prepared and assayed as described by Darras *et al.* (1992). Final incubations were in a total volume of 200 µl. For DI activity, final incubation mixtures contained 1 µM reverse T_3 (rT_3), 50 000 c.p.m./tube [3′,5′-^{125}I]rT_3 (Amersham Life Science, Inc., Arlington Heights, IL, USA), 100 µg/ml microsomal liver protein, 2 mM EDTA, and 5 mM dithiothreitol (DTT), and were incubated for 30 min at 37 °C. For DIII activity, incubations contained 1 nM T_3, 200 000 c.p.m./tube [3′-^{125}I]T_3 (Amersham Life Science, Inc.), 1 mg/ml liver microsomal protein, 1 µM rT_3, 0·1 mM propythiouracil, 2 mM EDTA and 50 mM DTT, and were incubated for 120 min at 37 °C.

Hepatic DIII Northern analysis

Chicken type I and type III iodothyronine deiodinases have been cloned and sequenced by Van der Geyten *et al.* (1997). [α-^{32}P]UTP-labeled cRNA probes were generated with the Ampliscribe T3 Transcription System (Epicentre Technologies, Biozym, Landgraaf, The Netherlands) and Northern analysis was conducted exactly

as described previously (Van der Geyten *et al.* 1999*a*). Briefly, total liver RNA was isolated with the RNAgents total RNA isolation system (Promega, Madison, WI, USA), separated on 1% formaldehyde-agarose gels (20 μg/lane) and transferred onto Hybond-N$^+$ membranes (Amersham Life Science, Inc.). Blots were hybridized at 57 °C (DI) or 66 °C (DIII) with cRNA probes in NorthernMax hybridization buffer (Ambion, Sanbio, Uden, The Netherlands). Blots were washed twice for 15 min each time at room temperature with 1 × SSC– 0·1% SDS, and twice for 1 h each time at 68 °C with 0·1 × SSC–0·1% SDS. Autoradiographs were generated from hybridized blots and scanned to determine density of DIII mRNA bands. Ethidium bromide-stained gels were similarly scanned to verify total RNA applied. Data are expressed as DIII mRNA signal density/total RNA signal density.

Statistical analyses

Percentage organ data were transformed to arcsin square root prior to statistical analysis. Plasma GH data displayed heterogeneity of variance, and were transformed to natural log scale prior to statistical evaluation. Dose–response data were analyzed by regression analysis and linear, quadratic and cubic functions tested for each response. Initially, each response was tested for a treatment × experiment replicate interaction, which was not significant for any measurement, therefore experiments were pooled and the replicate was dropped from the model.

For informational purposes, arithmetic, least squares means (LSM) ± standard error of the LSM are provided in figures and tables for all data, and any necessary transformations that were used are indicated.

Results

Arithmetic mean plasma GH concentrations for each dosage group are indicated in Fig. 1. Individual values within a dosage fell within a narrow range and did not overlap between groups. Natural log-transformed data followed a third order model as a function of GH dosage ($R^2 = 0·89$; Fig. 1).

Voluntary FI in GH-infused birds began to decline relative to controls at 100 μg/kg BW per day GH (7%), but was significantly reduced (19%; $P < 0·05$) only at the highest dosage (200 μg/kg BW per day). (See Wang *et al.* (2000) for complete profile.)

BW gain for GH-infused birds over the 7-day infusion period was reduced an average of 25% in comparison to controls ($P < 0·05$), with a tendency towards a dose-dependent decrease in weight gain (Table 1) that did not reflect FI responses as described above. Total (left plus right) kidney weight was increased an average of 14% for all GH-treated birds versus controls ($P < 0·05$), but this
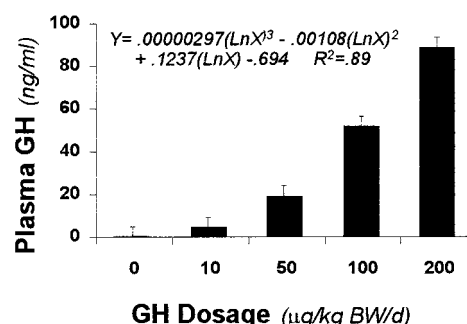

Figure 1 Mean plasma GH concentrations (ng/ml) in 8-week-old, female broiler chickens intravenously infused in a pulsatile pattern for 7 days with cGH at 0, 10, 50, 100 or 200 μg/kg BW per day. Daily doses were equally divided into sixteen 50 μl pulses each infused over a 2-min period every 90 min for a total of 0·8 ml/bird per day. Blood samples were collected by brachial venipuncture on the final day of infusion, immediately following completion of a pulse, and represent the peak plasma concentration of the hormone achieved. Values are LSM ± S.E. of the LSM ($n = 9$–10 birds/dosage). Ln = natural log.

response was again not dose-dependent (Table 1). Total kidney weight as a percentage of final body weight was increased in a dose-dependent manner with GH dosage, with arcsin square root transformed data following a linear function (Fig. 2). Other measures of somatic growth were not significantly altered by GH infusion, including weight of potential GH target organs such as the liver and spleen, longitudinal bone growth (tibiotarsal length), and adipose tissue deposition (Table 1). There were no significant differences in any somatic measures between control birds and birds pair-fed to the reduced level of FI of GH-infused birds (Table 2).

Plasma triacylglyceride concentrations were reduced approximately 32% ($P < 0·05$) with GH infusion, but this response was not dose-dependent (Table 3). Plasma glucagon, insulin, uric acid, IGF-I and IGF-II concentrations, muscle IGF-I content, and hepatic DI activity were not significantly altered by GH infusion (Table 3), nor were any plasma hormone or metabolite concentration, tissue IGF-I content, or hepatic iodothyronine deiodinase activities in pair-fed groups in comparison to controls (Table 4). Liver IGF-I content was significantly increased in a dose-dependent manner with GH infusion (Fig. 3).

The most dramatic, GH dose-dependent tissue response was a reduction in breast muscle mass, which followed a curvilinear (quadratic) function, with a maximal reduction of 12% after 7 days of infusion at the 100 μg/kg BW per day dosage (Fig. 4). Percentage breast muscle was similarly reduced with GH infusion, with arcsin square root-transformed data decreased in a linear fashion with GH dosage (Fig. 2). The dose-dependent reduction in breast muscle mirrored a curvilinear increase in plasma T_3 concentrations, and a decrease in hepatic DIII activity,

Table 1 Somatic measures[1] following 7 days of pulsatile i.v. infusion of vehicle or cGH to 8-week-old, female broiler-strain chickens

	cGH dosage (µg/kg BW per day)					Overall GH response[2]
	0 (*n*=9)	10 (*n*=9)	50 (*n*=10)	100 (*n*=10)	200 (*n*=10)	
Measured response						
7-day net BW gain						
(g)	485 ± 30·6	383 ± 30·6	388 ± 29·1	345 ± 29·4	330 ± 29·7	361 ± 14·7*
(% of initial BW)	20·4 ± 1·38	15·8 ± 1·38	16·2 ± 1·31	14·2 ± 1·33	13·8 ± 1·34	15·0 ± 0·66*
Kidneys						
(g)	19·1 ± 0·64	21·1 ± 0·64	21·4 ± 0·61	22·2 ± 0·61	22·3 ± 0·62	21·8 ± 0·31*
Liver						
(g)	63·4 ± 2·88	56·8 ± 2·88	64·5 ± 2·74	67·1 ± 2·76	67·5 ± 2·79	—
(% of final BW)	2·2 ± 0·10	2·0 ± 0·10	2·3 ± 0·09	2·4 ± 0·09	2·4 ± 0·09	—
Spleen						
(g)	4·6 ± 0·65	5·5 ± 0·35	4·3 ± 0·33	5·0 ± 0·34	4·8 ± 0·34	—
(% of final BW)	0·16 ± 0·012	0·19 ± 0·012	0·15 ± 0·012	0·18 ± 0·122	0·17 ± 0·012	—
Abdominal fatpad						
(g)	55·9 ± 4·94	50·3 ± 4·95	52·8 ± 4·70	48·4 ± 4·74	43·6 ± 4·79	—
(% of final BW)	1·9 ± 0·17	1·8 ± 0·17	1·9 ± 0·16	1·7 ± 0·16	1·6 ± 0·16	—
Skeletal muscle mass						
Left femur						
(g)	113·8 ± 3·37	116·7 ± 3·37	111·8 ± 3·20	109·6 ± 3·23	115·3 ± 3·26	—
(% of final BW)	3·9 ± 0·11	4·2 ± 0·11	4·0 ± 0·11	4·0 ± 0·11	4·2 ± 0·11	—
Left tibiotarsus						
(g)	90·0 ± 2·04	89·1 ± 1·94	83·7 ± 1·87	81·0 ± 1·88	80·9 ± 1·90	—
(% of final BW)	3·1 ± 0·08	3·2 ± 0·07	3·0 ± 0·07	2·9 ± 0·07	2·9 ± 0·07	—
Tibiotarsal length						
(mm)	120·7 ± 1·19	121·2 ± 1·26	121·0 ± 1·13	122·3 ± 1·14	120·9 ± 1·15	—

[1]LSM ± S.E. of the LSM. Tissue data expressed as a percentage of final BW (e.g. organs, muscle and adipose tissue) were converted to arcsin square root prior to statistical analysis and significant differences, where indicated, are based on the transformed data. However, untransformed means are provided above for informational purposes.
[2]For data indicated, there was no dose–response to GH, therefore the main effect of treatment (GH versus control) was evaluated and the overall (mean) GH response is presented.
*Significantly different from control (*P*<0·05).

which were also maximal at the 100 µg/kg BW per day dosage (108% increase for T_3; 72% reduction in DIII activity) (Fig. 4). Plasma T_4 was decreased with GH infusion, following a third order function, and this decrease was also maximal (49%) at the 100 µg/kg BW per day dosage (Fig. 4). Hepatic DIII mRNA expression was reduced 44% (*P*<0·05) by GH at the maximally effective dosage (100 µg/kg BW per day) (Table 5), whereas DIII message was increased (29%; *P*<0·05) with feed restriction (pair–feeding) (Table 5). These results correspond to previous findings by Darras *et al.* (1995) and Van der Geyten *et al.* (1999*b*).

Plasma metabolites that were significantly altered by GH infusion in a dose-dependent manner were plasma NEFA and glucose concentrations, which were increased linearly with GH dosage (Fig. 5).

Breast muscle free 3-methyl histidine at the GH dosage that resulted in the maximal reduction in breast muscle mass (100 µg/kg BW per day dosage) was not significantly different from the control group (Table 5). Breast muscle

free 3-methyl histidine content was also not significantly different between *ad libitum* fed controls and birds pair-fed to the reduced level of FI of this GH dosage group (Table 5).

Discussion

An objective of the present work was to better define GH-induced alterations in the pathway of thyroid hormone metabolism that would help to elucidate GH action in the chicken. In interpreting the results of this study, recognition of the basic elements of this pathway and their relative expression in the chicken are relevant. The metabolically active form of thyroid hormone, 3,3′,5-tri-iodo-L-thyronine (T_3), is derived from extrathyroidal (in particular, hepatic) outer ring deiodination (5′D) of T_4 by DI and DII, whereas inactivation of T_4 to rT_3, and of T_3 to di-iodothyronine by inner ring deiodination (5D) is catalyzed by DIII and DI respectively (Leonard & Visser
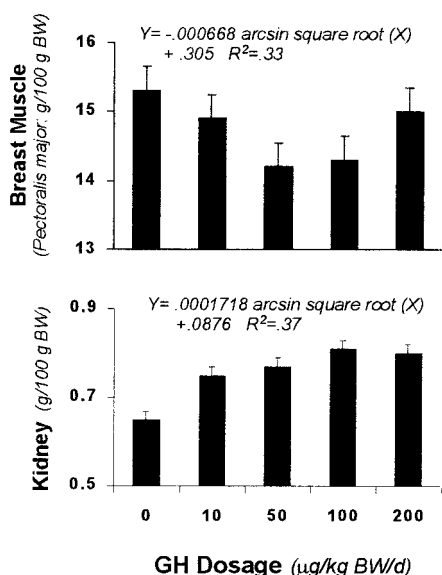
Figure 2 Breast muscle and kidney weights as a percentage of final BW in 8-week-old, female broiler chickens intravenously infused in a pulsatile pattern for 7 days with cGH at 0, 10, 50, 100 or 200 µg/kg BW per day. Tissues were collected on the final day of infusion, immediately following completion of a pulse. Values are LSM ± S.E. of the LSM (*n*=9–10 birds/dosage).

1986). Chick embryo liver reportedly contains DI, as well as considerable amounts of DIII, but little or no DII (for review see Kuhn *et al.* 1993). Acute administration of GH decreases hepatic 5 DIII activity in young chickens, which decreases peripheral T_3-degrading activity and, ultimately, increases circulating T_3 concentrations, but appears to have no effect whatsoever on the amount of hepatic DI (Darras *et al.* 1992, 1993). In chick embryos, a single GH injection was shown to effect the decrease in 5 DIII at a pretranslational level (Van der Geyten *et al.* 1999*a*).

The responses in plasma thyroid hormone and hepatic deiodinase activities in the present study extend previously published findings to chronic administration of GH in late post-hatch chickens. The present data also confirm that at least one mechanism by which GH elevates circulating T_3 is a dramatic reduction in T_3 degradation. Given that hepatic DI was not significantly altered by GH, the observed, co-ordinated decrease in plasma T_4 could be due to feedback inhibition of pituitary thyrotropin (TSH) secretion, as a result of elevated plasma T_3. Increased circulating T_3 in response to GH is observed in the human and other species, but often circumstantially assumed to result from increased conversion of T_4 (deLuze & LeLoup 1984, Wolf *et al.* 1989, Moller *et al.* 1992, Jorgensen *et al.* 1994, Johansson *et al.* 1996).

Because of the precise control possible with microprocessor-controlled, intravenous delivery in the present study, circulating concentrations of GH achieved over the full dose–response curve were extremely uniform

within dosage groups, and non-overlapping between groups. This enhanced definition of the response curves and confidence in the profile obtained for each response. What is most apparent from the present data, and previously unrecognized, is that GH not only failed to enhance overall body growth at any dosage, but the nature of the response was negative. This was particularly clear from the profile for breast muscle deposition (reduced net muscle mass versus controls). Interestingly, leg muscles for GH-treated birds in the present study were not reduced similarly to breast muscle, which may reflect differences in sensitivity of muscle fiber types. Chicken breast muscle is composed almost exclusively of white, IIB fast glycolytic fibers, whereas leg muscles reflect both type I and II red, slow oxidative fiber types (Aberle & Stewart 1983).

The loss of breast muscle mass in the present study would appear to conflict with documented anabolic effects of exogenous GH demonstrated in other species (with the exception of the guinea pig, as mentioned earlier). However, the response becomes rational in light of closely mirrored responses observed in thyroid hormone metabolism (in particular, hepatic DIII and plasma T_3), which suggest that this negative response is secondary to a GH-induced hyper-T_3 state, and not a direct, primary GH lesion. Net loss of muscle protein occurs in human patients with high serum T_3 (Hasselgren *et al.* 1984, Morrison *et al.* 1988) and in experimental models of hyperthyroidism (Carter *et al.* 1980), and supplemental T_3 reduces body weight gain in chickens (May 1980, Bowen *et al.* 1987, Tixier-Boichard *et al.* 1990).

Skeletal muscle mass is a function of the net balance in components of muscle protein turnover. In the present study, muscle 3-methyl histidine, regarded as an indicator of myofibrillar protein breakdown, was not increased in birds exhibiting the maximal degree of reduced muscle deposition (at the 100 µg/kg BW per day GH dosage). This suggests that the decreased muscle mass was not a function of increased muscle protein catabolism. Morrison *et al.* (1988) also failed to detect an increase in muscle 3-methyl histidine efflux in human patients with thyrotoxicosis and net loss of skeletal muscle protein. In fact, in the latter study, evidence was provided of reduced muscle protein breakdown as well as reduced synthesis rates, which led the authors to conclude that the net loss of protein in a hyper-T_3 state reflects more a decrease in muscle protein synthesis, rather than increased muscle proteolysis. As muscle protein synthesis rates were not determined in the present study, this cannot be confirmed for the GH-induced hyper-T_3 state of our birds.

It is unlikely that the reductions in breast muscle mass with GH infusion were due to GH-induced decreases in FI. Although birds infused with the highest dosage of GH (200 µg/kg BW per day) exhibited significantly reduced FI (19%), this response was not dose-dependent, as were the reductions in breast muscle mass. The latter coincided with increases in plasma T_3 concentrations over the full

Table 2 Somatic measures[1] following 7 days of pulsatile i.v. infusion of vehicle to 8-week-old, female broiler-strain chickens fed *ad libitum* (control) or restricted to the reduced level of voluntary feed consumption of birds infused with 100 or 200 µg cGH/kg BW per day (pairfed-100, pairfed-200)

| | Treatment group[2] | | |
	Control (n=9)	Pairfed-100 (n=9)	Pairfed-200 (n=10)
Measured response			
7-day net BW gain			
(g)	442 ± 19·0	489 ± 18·8	442 ± 20·0
(% of initial BW)	18·4 ± 0·80	20·1 ± 0·79	18·3 ± 0·84
Kidneys			
(g)	18·9 ± 0·46	19·2 ± 0·45	18·2 ± 0·48
(% of final BW)	0·66 ± 0·016	0·66 ± 0·015	0·64 ± 0·016
Spleen			
(g)	7·0 ± 0·62	6·6 ± 0·61	5·3 ± 0·65
(% of final BW)	0·24 ± 0·021	0·23 ± 0·021	0·19 ± 0·022
Liver			
(g)	69·5 ± 4·00	70·3 ± 3·96	64·8 ± 4·23
(% of final BW)	2·4 ± 0·13	2·4 ± 0·13	2·3 ± 0·14
Abdominal fatpad			
(g)	56·1 ± 4·9	51·1 ± 4·9	53·4 ± 5·2
(% of final BW)	2·0 ± 0·17	1·7 ± 0·17	1·9 ± 0·18
Skeletal muscle mass			
Left femur			
(g)	115·4 ± 3·06	112·5 ± 3·03	112·8 ± 3·23
(% of final BW)	4·0 ± 0·11	3·9 ± 0·11	4·0 ± 0·11
Left tibiotarsus			
(g)	85·6 ± 2·44	86·5 ± 2·42	89·3 ± 2·58
(% of final BW)	3·0 ± 0·09	3·0 ± 0·09	3·1 ± 0·10
Breast			
(g)	432 ± 9·9	418 ± 9·8	429 ± 10·4
(% of final BW)	15·1 ± 0·33	14·4 ± 0·33	15·0 ± 0·49
Tibiotarsal length			
(mm)	124·3 ± 1·12	122·5 ± 1·11	120·8 ± 1·18

[1]LSM ± S.E. of the LSM.
[2]There were no significant differences among treated groups for any somatic measures ($P>0.05$).

dose–response curve, with both responses maximal at 100 µg/kg BW per day GH. Also, birds pair-fed to the level of reduced FI of GH-infused birds did not exhibit significant reductions in body weight gain or breast muscle mass in comparison to controls.

GH infusion did increase kidney weight, likely reflecting the organ-specific nature of a hyper-T_3 state, which is generally catabolic for skeletal muscle, but not for critical visceral organs. For example, treatment with T_3 from birth until weaning selectively enhanced all measures of cardiac growth, but significantly reduced skeletal muscle (gastrocnemius) total DNA, RNA, and total and myofibrillar protein content in rats (Moussavi 1990).

The underlying mechanism(s) by which net skeletal muscle deposition is reduced in a hyper-T_3 state is (are) not known. It is generally accepted that the anabolic effects of GH on skeletal muscle are mediated via the actions of IGF-I, and at least partially by IGF-I produced by the liver and acting in an endocrine manner. Plasma IGF-I concentrations are partially but not totally GH-dependent in the chicken, as levels are reduced to 30–50% of normal in hypophysectomized birds and to approximately one-third normal levels in sex-linked dwarf chickens, the latter which exhibit impaired GH receptor function (for review see Vasilatos-Younken 1999). All indications are that the effects of IGF-I upon satellite cell proliferation and, thereby, postnatal muscle growth, are similar in chickens to those in mammals (for review see Vasilatos-Younken 1999). It is therefore relevant that GH failed to elevate circulating IGF-I concentrations at any dosage (as determined by a homologous RIA that ensured IGFBPs were not an interfering factor (McMurtry *et al.*

Table 3 Plasma hormones and metabolites, skeletal muscle IGF-I concentrations, and hepatic type I iodothyronine deiodinase activity[1] following 7 days of pulsatile i.v. infusion of vehicle or cGH to 8-week-old, female broiler-strain chickens

	cGH dosage (μg/kg BW per day)					Overall GH response[2]
	0 (*n*=9)	10 (*n*=9)	50 (*n*=10)	100 (*n*=10)	200 (*n*=10)	
Plasma concentration						
Glucagon (pg/ml)	145·1 ± 11·79	161·0 ± 11·18	153·0 ± 10·8	131·4 ± 10·8	148·5 ± 11·5	—
Insulin (ng/ml)	2·39 ± 0·364	1·77 ± 0·346	1·88 ± 0·332	2·84 ± 0·332	2·37 ± 0·355	—
Uric acid (mg/dl)	7·3 ± 0·82	4·1 ± 0·78	5·6 ± 0·75	4·8 ± 0·75	6·4 ± 0·80	—
Trigylcerides (mg/dl)	116 ± 6·67	73·6 ± 6·32	88·3 ± 6·08	77·5 ± 6·08	76·1 ± 6·5	78·9 ± 3·27*
IGF-I (ng/ml)	10·9 ± 1·02	12·7 ± 0·97	12·4 ± 0·9	11·2 ± 0·9	14·1 ± 1·00	—
IGF-II (ng/ml)	22·8 ± 1·05	25·9 ± 1·00	24·6 ± 0·96	23·6 ± 0·96	25·6 ± 1·03	—
Tissue IGF-I concentration (pg/mg protein)						
Pectoralis major	301·9 ± 120·5	138·2 ± 120·5	322·8 ± 114·3	98·9 ± 114·3	291·8 ± 114·3	—
Gastrocnemius	607·8 ± 169·9	868·0 ± 169·9	1024·3 ± 161·2	736·9 ± 161·2	944·3 ± 161·2	—
Hepatic iodothyronine deiodinase activity						
DI (pmol rT$_3$ deiodinated/mg microsomal protein per min)	117·4 ± 6·74	103·1 ± 6·74	106·0 ± 6·39	106·2 ± 6·39	99·9 ± 6·39	—

[1]LSM ± S.E. of the LSM.
[2]For data indicated, there was no dose–response to GH, therefore the main effect of treatment (GH versus control) was evaluated and the overall (mean) GH response is presented.
*Significantly different from control (*P*<0·05).

Table 4 Plasma hormones and metabolites, tissue IGF-I concentrations, and heaptic iodothyronine deiodinase activities[1] following 7 days of pulsatile i.v. infusion of vehicle to 8-week-old, female broiler-strain chickens fed *ad libitum* (control) or restricted to the reduced level of voluntary feed consumption of birds infused with 100 or 200 μg cGH/kg BW per day (pairfed-100, pairfed-200)

	Treatment group		
	Control (*n*=9)	Pairfed-100 (*n*=9)	Pairfed-200 (*n*=10)
Plasma concentration			
Glucagon (pg/ml)	147·3 ± 14·05	127·7 ± 14·05	142·6 ± 14·90
Insulin (ng/ml)	1·91 ± 0·386	2·16 ± 0·386	2·76 ± 0·410
IGF-I (ng/ml)	8·2 ± 0·80	8·4 ± 0·80	9·1 ± 0·85
IGF-II (ng/ml)	23·7 ± 0·92	23·3 ± 0·92	25·1 ± 0·97
Glucose (mg/dl)	261·7 ± 8·31	261·8 ± 8·31	256·5 ± 8·81
NEFA (μEq/l)	147·0 ± 11·63	158·9 ± 11·63	154·8 ± 12·33
Trigylcerides (mg/dl)	91·8 ± 8·08	102·6 ± 8·08	87·3 ± 8·57
Uric acid (mg/dl)	6·8 ± 0·94	7·5 ± 0·94	8·0 ± 1·00
Tissue IGF-I concentration (pg/mg protein)			
Liver	ND[2]	0·8 ± 1·83	4·5 ± 1·94
Pectoralis major	174·1 ± 129·3	299·3 ± 129·3	197·4 ± 137·1
Gastrocnemius	542·3 ± 163·72	1048·9 ± 163·72*	487·1 ± 173·7
Hepatic iodothyronine deiodinase activity			
DI (pmol rT$_3$ deiodinated/mg microsomal protein per min)	93·2 ± 8·41	96·4 ± 8·41	89·8 ± 8·92
DIII (fmol T$_3$ deiodinated/mg microsomal protein per min)	0·61 ± 0·408	0·83 ± 0·41	1·51 ± 0·43

[1]LSM ± S.E. of the LSM.
[2]Not detectable.
*Significantly different from control group (*P*<0·05).
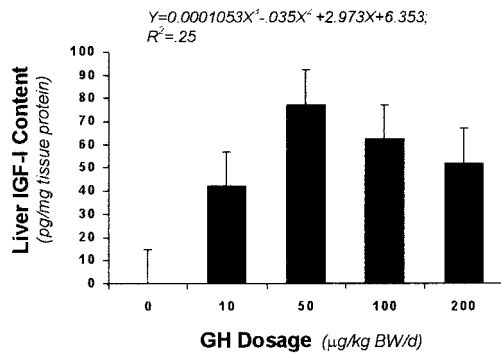
$$Y=0.0001053X^3-.035X^2+2.973X+6.353;\ R^2=.25$$

Figure 3 Liver IGF-I protein concentration (pg/mg tissue protein) in 8-week-old, female broiler chickens intravenously infused in a pulsatile pattern for 7 days with cGH at 0, 10, 50, 100 or 200 μg/kg BW per day. Tissues were collected on the final day of infusion, immediately following completion of a pulse. Values are LSM ± S.E. of the LSM (n=9–10 birds/dosage).

1994)), despite evidence that early events in the GH signaling pathway were intact (tyrosine phosphorylation of JAK2 kinase; Zhou *et al.* 1998) and despite a significant, dose–dependent increase in hepatic IGF-I protein content, which increased from virtually undetectable levels to approximately 80-fold higher at the maximally effective GH dosage. These would indicate that GH signal transduction is intact to the point of stimulating IGF-I synthesis in liver, but that release into the circulation is somehow impaired.

There is evidence that thyroid hormones may be important regulators of IGF-I, and affect synthesis, release and/or bioactivity of the growth factor. Methimazole-induced hypothyroidism decreased circulating IGF-I levels in broilers (Decuypere *et al.* 1987). Thomas *et al.* (1993) reported similar serum IGF-I concentrations, but a 70% reduction in serum IGF-I bioactivity and reduced body weight in hyperthyroid rats compared with control animals. If such a reduction in bioactivity occurred in the present study, a reduction in skeletal muscle protein synthesis would be conceivable, despite no significant alteration in circulating, immunoassayable IGF-I concentrations. Alternatively, T_3 decreased IGF-I released by neuronal cells in culture (Binoux *et al.* 1985), and circulating IGF-I was reduced in chickens administered supplemental T_3 (Vasilatos-Younken *et al.* 1997, Tixier-Boichard *et al.* 1990). Wolf *et al.* (1989) demonstrated in hypophysectomized rats that thyroid hormones had major effects on GH-stimulated IGF-I synthesis and secretion, with the pattern depending upon prior exposure to GH and/or thyroid hormones, and the temporal relationship between GH and thyroid hormone administration. In the latter study, a single injection of T_3 alone had little effect on circulating IGF-I levels, whereas a single injection of T_3 plus GH slightly increased IGF-I above the level achieved with GH alone, and a single
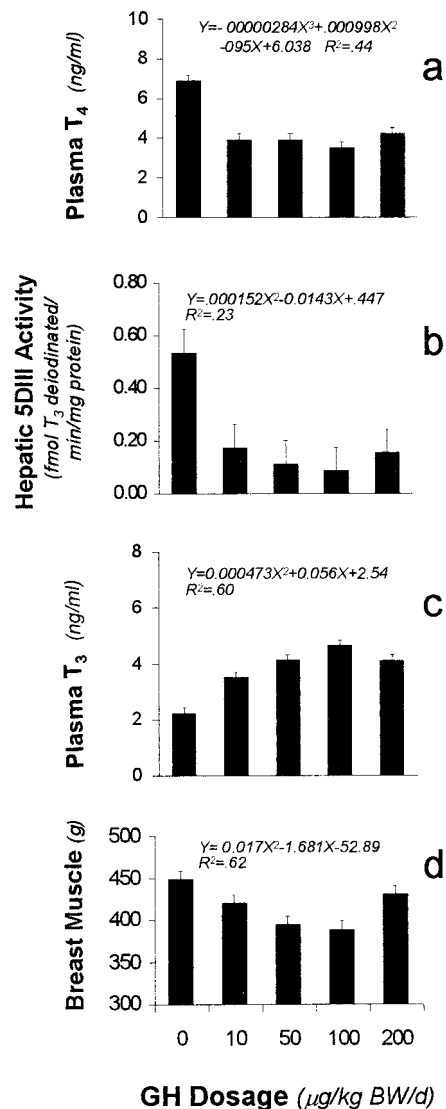
$$Y=-.00000284X^3+.000998X^2-.095X+6.038\quad R^2=.44$$

$$Y=.000152X^2-0.0143X+.447\ R^2=.23$$

$$Y=0.000473X^2+0.056X+2.54\ R^2=.60$$

$$Y=0.017X^2-1.681X-52.89\ R^2=.62$$

Figure 4 Mean (a) plasma T_4 (ng/ml), (b) hepatic DIII deiodinase activity (5 DIII; fmol T_3 deiodinated/mg liver microsomal protein per min), (c) plasma T_3 (ng/ml), and (d) breast muscle mass (g) in 8-week-old, female broiler chickens intravenously infused in a pulsatile pattern for 7 days with cGH at 0, 10, 50, 100 or 200 μg/kg BW per day. Blood and tissue samples were collected on the final day of infusion, immediately following completion of a pulse. Values are LSM ± S.E. of the LSM (n=9–10 birds/dosage).

injection of T_3 following 10 days of GH treatment substantially lowered IGF-I versus GH-treated, pre-T_3 levels (Wolf *et al.* 1989). As plasma T_3 was increased at every GH dosage in the present study, it is not known whether GH enhancement in the absence of increased T_3 would successfully effect an increase in circulating IGF-I. It is reasonable to speculate, however, that the apparent inability of exogenous GH to increase circulating IGF-I concentrations in immature, growing chickens, as noted

Table 5 Hepatic type III iodothyronine deiodinase mRNA and skeletal muscle (pectoralis major) free 3-methyl histidine concentrations[1] following 7 days of pulsatile i.v. infusion of vehicle (control) or 100 µg cGH/KG BW per day to 8-week-old, female broiler-strain chickens fed *ad libitum*, or following 7 days of pulsatile i.v. infusion of vehicle to birds fed *ad libitum* (control) or restricted to the reduced level of voluntary feed consumption of birds infused with 100 µg cGH/kg BW per day (pairfed-100)

	n	Hepatic DIII mRNA (DIII mRNA OD/total RNA OD)	Muscle free 3-methyl histidine (nmol/g tissue dry matter)
Infusion treatment			
Vehicle control (0 cGH)	10	34·2 ± 1·34	74·4 ± 5·21
100 µg cGH/kg BW per day	11	19·2 ± 1·28*	69·8 ± 4·66
Dietary treatment			
Control (fed *ad libitum*)	5	39·2 ± 2·18	70·5 ± 4·82
Pairfed-100	8	50·7 ± 1·72*	67·5 ± 5·11

[1]LSM ± S.E. of the LSM.
*Significantly different from control ($P<0·05$).

in this and previous studies (for review see Vasilatos-Younken 1999), is secondary to GH-induced hyperthyroidism.

The metabolic effects of GH are considered direct actions of the hormone, and evidence of such effects was apparent in GH-infused birds in this study. These included a dose-dependent increase in plasma NEFA concentrations, which was linear over the entire GH dosage range, and reflects the direct, lipolytic nature of cGH, documented previously *in vitro* (Campbell & Scanes 1985, 1987). Although evidence for a hyperglycemic

Figure 5 (a) Plasma NEFA (µEq/l) and (b) glucose (mg/dl) concentrations in 8-week-old, female broiler chickens intravenously infused in a pulsatile pattern for 7 days with cGH at 0, 10, 50, 100 or 200 µg/kg BW per day. Blood samples were collected on the final day of infusion, immediately following completion of a pulse. Values are LSM ± S.E. of the LSM (*n*=9–10 birds/dosage).

effect of GH was observed, this was in the absence of any significant increase in circulating insulin concentrations, consistent with previous studies from our laboratory with GH infused in a pulsatile pattern (Vasilatos-Younken *et al.* 1988). This may reflect a direct, gluconeogenic effect on the liver, but does not appear to elicit the diabetogenic response typically observed in mammalian species such as the human (Sonksen *et al.* 1993) and the pig (Etherton *et al.* 1993).

Finally, given the significant (44%) reduction in hepatic DIII mRNA in birds infused with the maximally effective (in terms of thyroid axis responses) GH dosage, a cohesive and reasonable pathway by which GH impacts on thyroid hormone metabolism and, ultimately, muscle growth in the chicken emerges. This pathway begins at the pretranslational level of control, with reduced mRNA expression of hepatic 5 DIII resulting in reduced DIII protein product, and reflected in a reduced level of enzyme activity. This decrease in DIII activity contributes to a reduction in the conversion of T_3 to its inactive form, thereby elevating circulating T_3 levels. Elevated T_3 results in TSH-mediated feedback inhibition of circulating T_4 concentrations, and impairment of hepatic IGF-I release, with attendant reduced net skeletal muscle deposition.

In summary, the present study clearly demonstrates that GH does in fact have significant biological effects in the chicken. Most importantly, it underlines the recognition that among these are profound metabolic actions that appear to predominate, and may limit the potential for GH to elicit positive, IGF-I-mediated skeletal muscle growth in the chicken.
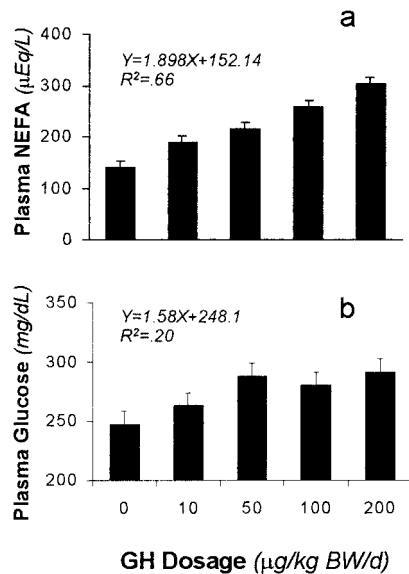
Acknowledgements

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